

STANDARD OPERATING PROCEDURES
DIVISION OF COMPARATIVE MEDICINE
UNIVERSITY OF SOUTH FLORIDA

SOP#: 457.1

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TITLE: **Germ-free and Gnotobiotic Mice Care and Use**
SCOPE: All Authorized Personnel
RESPONSIBILITY: Facility Manager and Technical Staff
PURPOSE: To Outline the Proper Procedures for Safely Caring for and Using
Germ-free & Gnotobiotic Mice

I. PURPOSE

1. To outline the proper procedures for safely and effectively conducting husbandry, handling and use of **germfree and gnotobiotic** (GF) mice housed in bioexclusion isolation individually ventilated

13. In addition, a **dated Integrator strip** must be **taped to the front** of each GF sterilization transport rack prior to sterilization and left in place when delivered to GF housing.
14. After IsoIVC-P autoclave sterilization, using gloves and sleeves that are saturate-sprayed with Oxivir-Tb, the center “indicator” IsoIVC-P is carefully removed and discarded, and the **Integrator strip dated**, recorded, and **taped to the front** of a remaining IsoIVC-P on the GF sterilization transport rack to indicate sterilization date of the delivered load.
15. The Biological **Indicator** from the “indicator” IsoIVC-P is set in the incubator, read and recorded per SOP 1002.
16. If more than one autoclave IsoIVC-P load is processed in a single day, loads must be distinguished by labeling Biological Indicators and Integrators (e.g., date-A, date-B, date-C).
17. In addition, for each autoclave sterilized load, the dated **autoclave printer strip is taped to the front** of the GF sterilization transport rack to document its completion of a GF sterilization cycle.
18. After removing the indicator IsoIVC-P, using gloves and sleeves that are saturate-sprayed with Oxivir-Tb, the GF sterilization transport racks of sterile IsoIVC-P are taken directly to GF isolation for **two-person entry**, see below.
19. **Each delivered** GF transport rack of IsoIVC-P must have both a **dated autoclave printer strip** and a **dated Integrator strip** that has changed in accordance with SOP 1002, **taped to the front** of one IsoIVC-P when delivered to GF housing, and left in place until IsoIVC-P use.
20. If Integrator strips or Biological Indicators after incubation have not changed according to SOP 1002 and SOP 1013, **notify** your supervisor and GF isolation staff immediately. Such supplies are **rejected** and returned to dirty side.
21. Inside GF isolation, each IsoIVC-P is left held tightly in place by spring-loaded runners, allowed to cool.
22. Each IsoIVC-P is then carefully **latched shut** by raising the closing clamps on each side, with the clamp tabs secured under the top rim of the box. The clamps are then pushed inwards, and the white safety buttons on each side are pushed downwards, **locked**. Ensure the **white tab** presses smoothly and becomes fully **down**.
23. Locked sterile IsoIVC-P are then placed on the GF housing rack for use, fully docked if the air handling unit AHU of the rack is actively pressurized and ventilating, but **not fully docked if the AHU is off**.

III.B. Autoclave Sterilization of Reservoir Bottles

1. Autoclaved RO drinking water is prepared in **reservoir bottles** in accordance with SOP 1006 Autoclave Sterilization and SOP 1013 Monitoring Steam Sterilization of Liquids except that the **GF liquid sterilization cycle** must be 122°C, 90 minutes, 2.45 psia vacuum (i.e., no pre- or post-pulse stages).
2. Baskets of reservoir bottles (i.e., Corning 1L glass bottles) are filled with RO drinking water, **capped loosely** with red high temperature-resistant caps, and are **Tyvek covered** over the top.
3. Filled reservoir RO water bottles are validated as sterile per SOP 1013 using a **MagnaAmp** indicator suspended inside one filled uncapped “indicator” bottle, and a **dated Integrator strip taped onto the top Tyvek cover of each basket** in each autoclaved load.
4. After sterilization, using gloves and sleeves saturate-sprayed with Oxivir-Tb,02 Tc -0.002 Tw 0.6

8. If additional consumable supplies, equipment, reagents or biologics are needed during study, they must be in **nonporous containers** (e.g., Nunc tubes, autoclave-

13. Do **not** use solvent containing solutions to clean the Qube control panel screen or front access door glass. The control panel screen will be damaged if cleaned with a solvent containing solution.
14. **Occupied IsoIVC-P** must **never** be left **undocked** from the bioexclusion housing rack, since each primary enclosure is a sealed unventilated microenvironment.
15. Plans for exterior bio-decontamination of occupied Iso-IVC inside the Qube must be finalized, and all supplies and equipment assembled in advance, prior to undocking an occupied Iso-IVC.

III.F. Qube System “Glove-box” Isolator

1. Whenever possible, **work in at least pairs**.
2. The Bioquell Qube is an aseptic workstation, a rigid “glove-box” isolator, with an integral HPV bio-decontamination system, which is loaded via the front access window.
3. The front access window is opened and shut by carefully pulling or pushing on the **rim of the glove ports**. Warning, **do not push** on the sides of the window as this could cause damage.
4. The Qube system has 2 large chambers, a hydrogen peroxide vapor (**QHPV**) module, and an extension (**QEXT**) module. Each of these 2 larger chambers is flanked by a smaller, material transfer device (**QMTD**) used for exiting processed material.
5. The complete Qube system is controlled via a control panel on the QHPV module.
6. The QEXT enables the operator to work on or store one load while simultaneously HPV decontaminating another load in the QHPV.
7. The QMTD is used to transfer the completed load out of the Qube system.
8. Qube system interior lighting is “white”, but changes to “blue” during a HPV cycle.
9. When not in use, to ensure diurnal lighting of GF isolation housing, the interior lights of both the QHPV and the QEXT should be **switched off**.
10. During **bio-decontamination mode**, the QHPV provides assurance of a 6-log microbial reduction of the load placed inside the QHPV module.
11. Bio-decontamination can be of a load placed inside the QHPV (i.e., **QHPV loaded cycle**), or of the empty Qube system with all interior doors open (i.e., **System empty cycle**).
12. During “use3yw 1.639 0 Td [(D) Twa[(st)0.7(em)-0.7()]TJ 0 Tc 0 Tw -3124h C -41.213n

requirements of **ISO 14644-1 Class 5** (equivalent to EU cGMP Grade A and US FED STD 209E Class 100).

13. During “use” or processing mode, the Qube system maintains an **airflow rate** of **573 m³/h** and chamber **pressure set points** of **75 Pa** for the QHPV, **50 Pa** for the QEXT, and **36 Pa** for each of the QMTDs (i.e., a positive pressure gradient from the QHPV to the room).
14. During a bio-decontamination cycle a pressure setpoint of **50 Pa** is maintained.
15. The bio-decontamination **QHPV loaded cycle** (i.e., either a QHPV loaded cycle or a system empty cycle) is a four-stage process that includes (1) **conditioning**, when the vaporizer increases to operating temperature, (2) **gassing**, when the hydrogen peroxide liquid is drawn from its bottle, flash vaporized and distributed around the chamber(s), dew point is reached and micro-condensation forms on Qube interior surfaces, (3) **dQ Tc (-)Ej(f10-00-0.00 [c-3] (1) 77 10 0) on dra 0001 rawn] 6B910.197HFFje)EMOV(z)JT**

24. To **open a side door** between a chamber, depress the footswitch located below it

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13. Work carefully, systematically, comparably to guidance provided in SOP 400 Rodent Husbandry, using reservoir bottles to fill drinking water bottles, transferring mice to a clean environment, and reporting any concerns.
14. Daily observations are recorded on the Room Status Sheet within the Room Log Book, with health surveillance occurring twice per day on weekdays and daily on weekends.
15. Observations and communications should be noted on the Room Status Sheet **CMDC #41** and the Animal Health and Environmental Concern Form **CMDC #77**.
16. Refer to SOP #006, entitled Animal Health and Environmental Surveillance, for specific procedures of reporting and recording health concerns.
17. IsoIVC-P is changed as a unit. IsoIVC-P that is excessively soiled may be changed at the discretion of the animal care technician. IsoIVC-P housing gnotobiotic mice are routinely changed every two weeks. IsoIVC-P housing germfree mice are routinely changed every two weeks, but can be extended to three or four weeks at the discretion of the veterinarian, due to the absence of microbiota-effects on primary enclosure environmental quality.
18. After transfer of mice to clean IsoIVC-P, **soiled IsoIVC-P are not to be disassembled** within the Qube, but left assembled and clamped, removed from the room fully intact, and taken to cage wash for breakdown and sanitation.
19. After completion of husbandry tasks, all IsoIVC-P must be **clamped shut** prior to opening the Qube's front access window and returning occupied IsoIVC-P to the bioexclusion housing rack.
20. Bioexclusion rack air handling unit (AHU) **prefilters** must be changed out every 2 weeks or as needed, carefully bagged and removed from the room, and this recorded on the Room Status Sheet .
21. Bioexclusion racks must be changed out every 6 months, and this recorded on the Room Status Sheet .
22. Complete and record other room duties, which are comparable to those specified in SOP 400 Rodent Husbandry.

NOTE: If at any time there is a medical emergency (e.g., moribund, dystocia, bleeding, irretractable seizures, lethargy, dehydration, etc.), the clinical veterinarian is to be notified immediately.

III.H. Taconic Shipper Receipt Using the Qube System

1. Whenever possible, **work in at least pairs**. Three staff are helpful for receipt of GF mice delivered inside a Taconic shipper.

2. The QHPV has an integrated HPV decontamination system, and is loaded via the front access window. On the control panel, activate the “open front access window” button, gently push in on the **rim of the sleeve port** until a “click” is heard, the window seal will deflate, the window will release, then gently pull on the rim of the sleeve port to open.
3. Load the QHPV with items to be decontaminated, ensuring good gassing presentation, and that **occluded surface areas** of the load are **minimized**.
4. Prior to closing the front access window, ensure sleeves are folded in the correct position, and that **gloves** are suspended by the magnet in the front window with glove fingers **fully extended**.
5. **Receiving** and unpacking **germ-free mice** delivered in a **single Taconic shipper requires 3 HPV cycles** to safely unpack, test, and (presumably) confirm the germ-free status of each shipper.
6. **Work flow** while unpacking the Taconic shipper proceeds from **left to right** from the **QHPV** into the **QEXT** into the right **QMTD**.
7. Confirm that **2-3 individuals** are scheduled for receipt, one working in the QHPV, one working in the QEXT, and preferably one working in the GF isolation room, supporting the others, including **recordkeeping**.
8. Confirm that **appropriate glove sizes** are in place in each of the chambers for the individuals scheduled to work in the QHPV and QEXT.
9. Staff assigned to **support work** in the GF isolation room wears **sleeves and gloves** saturate-sprayed with Oxivir-Tb, dried, then wiped dry with autoclave sterilized towels, and then with Steris 6% hydrogen peroxide wipes.
10. Staff working inside either of the **chambers** rely on the **sleeve and glove integral** to the QHPV or QEXT front access window.
11. **Before** the scheduled receipt of a Taconic shipper of germ-free mice, run (**1st cycle**) a **System Empty Cycle**.
12. Also, **before receipt**, confirm that all autoclaved equipment and supplies (e.g., Iso-IVC, reservoir bottles, Nunc specimen **tubes in an Iso-IVC**) required for receipt are on hand in GF isolation.
13. After completion of a system empty cycle, the QHPV, QEXT, and QMTDs are considered in **aseptic hold**.
14. Confirm on the control screen that a **green halo** surrounds the images of the QHPV, QEXT, and QMTDs, indicating that all remain in aseptic hold, in a **sealed, HPV-decontaminated** condition.
15. The **aseptic hold period** is the validated time that aseptic conditions are maintained in the QEXT displayed at the top of the control screen in **days and**

26. Using **flexible port caps**, securely **seal** the HEPA filtered vents of the Taconic shipper, then place it, a pair of **scissors**, and **forceps** into the QHPV.
27. The **shipper** should be **positioned** to the **QHPV left side**, so that after cycle completion static boxes can be passed through the middle inner door without

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Effective 085 Tds[822 Tds]

43. Simultaneously, while running the loaded QHPV cycle, **working in pairs**, one in the QHPV and one in the QEXT, the individual in the QEXT, **unclamps** all 3 Iso-IVC, **fills** all 3 Iso-IVC water bottles using the 3 red-capped reservoir bottles, **positions** all 3 unclamped Iso-IVC now ready to receive mice, and positions the Nunc tube in its rack for collecting a single specimen of two fresh fecal pellets to confirm germ-free status of the shipper. Tops can be **stacked** out of the way in the QEXT on the shelf or base wire grid, and Iso-IVC wire tops used to contain mice as germ-free trios are created.
44. After completion of this “3rd cycle”, the individual in the QHPV opens the Taconic shipper with the scissors, making a broad “**U**”-**shaped incision** through the clear plastic avoiding the covered HEPA filter on the top of the shipper, then folds open, the created plastic flap, making accessible the static cages containing germ-free mice.
45. Working in pairs, the individual in the QHPV opens the side door, and passes a cage of mice through to the individual in the QEXT. If male mice, one will need to be entered into each of the 3 Iso-IVC. If female mice, two mice will need to be entered into each Iso-IVC.
46. A single Nunc tube containing **specimens** of two fresh fecal pellets, preferably from different mice shipped in different static cages, is collected to confirm germ-free status of the shipper.
38. In sequence, the QHPV individual exits the 3 static boxes of mice, one by one, into the QEXT for the creation of breeding trios in each of the Iso-IVC. To make space in the QEXT, **emptied** static **boxes**, emptied water **reservoir bottles**, and the Nunc rack and **sealed tube** containing specimens can be **exited** via the **right QMTD** to the third individual working in GF isolation. Note, that there will be a **2-minute clean-up time** before the internal door of the QMTD can be opened again.
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2. Fecal pellets are collected fresh in sterile Eppendorf tubes, prior to entering bedding, and stored immediately at -20°C. Approximately 10 pellets should be collected for each slurry preparation.
3. All work for fecal slurry preparations should be done in a biosafety hood, with all sterile tools and sanitized surfaces.
4. Work surfaces are decontaminated with Oxivir-Tb before and after use and in between different flora samples.
5. Each unique fecal slurry requires a 100 um cell strainer, two 50 ml conical tubes, sterile pipette tips, pipette, 5-10 ml syringe or handheld electric pipette with 5 or 10 ml serological pipettes, sterile cryotubes, label maker, sterile PBS, and Mr. Frosty container for long term storage in -80°C. !Í 001 <21MCID 17 >>BDC rm